



CLEAN VERSION OF AMENDED SPECIFICATION PARAGRAPHS

METALLOPROTEINASE INHIBITORS FOR WOUND HEALING

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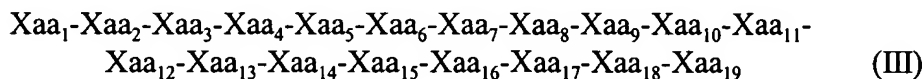
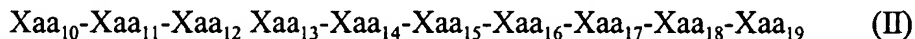
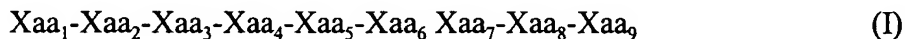
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At page 2, the amended paragraph beginning on line 24 and continuing on page 3:

The present invention is directed to peptide inhibitors of matrix metalloproteinases. These peptide inhibitors have amino acid sequences identical or related to the linking region spanning the two globular domains of matrix metalloproteinases. Several types of matrix metalloproteinases and their sequences are known, including matrix metalloproteinase-1, matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-4, matrix metalloproteinase-5, matrix metalloproteinase-6, matrix metalloproteinase-7, matrix metalloproteinase-8, and matrix metalloproteinase-9, matrix metalloproteinase-10, matrix metalloproteinase-11, matrix metalloproteinase-12, and matrix metalloproteinase-13. The invention contemplates inhibitors having amino acid sequences from the linking region of any of the matrix metalloproteinases. For example, peptide inhibitors of the invention can have amino acid sequences drawn from any region from about amino acid 70 to about amino acid 120 of the matrix metalloproteinase-2 sequence (SEQ ID NO:14), and analogous regions of all other matrix metalloproteinases.

At page 3, the amended paragraph beginning on line 9 and continuing on page 4 :

The invention provides peptides of any one of formulae (I), (II), (III):



wherein

Xaa₁, Xaa₄, and Xaa₆ are separately each apolar amino acids;

Xaa₂ is a basic amino acid;

Xaa₃ is a cysteine-like amino acid;

Xaa₅ is a polar or aliphatic amino acid;

Xaa₇ is an acidic amino acid,

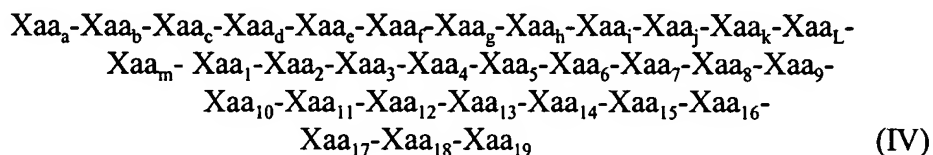
Xaa₈ is an aliphatic or polar amino acid;

Xaa₉ is an aliphatic, apolar or basic amino acid; and

Xaa₁₀ is a polar, acidic, basic or apolar amino acid;
 Xaa₁₁ is a polar or aromatic amino acid;
 Xaa₁₂ is a polar, basic, aliphatic or apolar amino acid ;
 Xaa₁₃ is an aromatic, aliphatic, polar or acidic amino acid;
 Xaa₁₄ is an aromatic, apolar or polar amino acid;
 Xaa₁₅ is an apolar or acidic amino acid;
 Xaa₁₆ is a basic, a polar or an apolar amino acid;
 Xaa₁₇ is a basic, a polar, an aliphatic, an apolar or an acidic amino acid;
 Xaa₁₈ is an apolar or an aliphatic amino acid;
 Xaa₁₉ is a basic or an aliphatic amino acid; and
 wherein the peptide is capable of inhibiting the activity of matrix metalloproteinase-1, matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-4, matrix metalloproteinase-5, matrix metalloproteinase-6, matrix metalloproteinase-7, matrix metalloproteinase-8, or matrix metalloproteinase-9, matrix metalloproteinase-10, matrix metalloproteinase-11, matrix metalloproteinase-12, and matrix metalloproteinase-13. In a preferred embodiment, the peptide can inhibit the activity of matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-7, matrix metalloproteinase-8, or matrix metalloproteinase-9.

At page 5, the amended paragraph at line 1 and continuing on page 6:

The invention also provides peptides of formula (IV) (SEQ ID NO:18):



wherein:

Xaa _a is proline;	Xaa ₁ is proline;
Xaa _b is glutamine or glutamic acid;	Xaa ₂ is arginine;
Xaa _c is threonine;	Xaa ₃ is cysteine;
Xaa _d is glycine;	Xaa ₄ is glycine;
Xaa _e is aspartic acid or glutamic acid;	Xaa ₅ is valine or asparagine;
Xaa _f is leucine;	Xaa ₆ is proline;
Xaa _g is aspartic acid;	Xaa ₇ is aspartic acid;
Xaa _h is glutamine or serine;	Xaa ₈ is valine or leucine;
Xaa _i is asparagine or alanine;	Xaa ₉ is alanine or glycine;
Xaa _j is threonine;	Xaa ₁₀ is asparagine or arginine;
Xaa _k is isoleucine or leucine;	Xaa ₁₁ is tyrosine or phenylalanine;

Xaa_L is glutamic acid or lysine;

Xaa_m is threonine or alanine;

Xaa_n is methionine;

Xaa_o is arginine;

Xaa_p is lysine or threonine;

Xaa₁₇ is lysine or aspartic acid;

Xaa₁₉ is lysine; and

Xaa₁₂ is asparagine or glutamine;

Xaa₁₃ is phenylalanine or threonine;

Xaa₁₄ is phenylalanine;

Xaa₁₅ is proline or glutamic acid;

Xaa₁₆ is arginine or glycine;

Xaa₁₈ is proline or leucine;

wherein the peptide is capable of inhibiting the activity of a metalloproteinase. For example, the metalloproteinase can be matrix metalloproteinase-1, matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-4, matrix metalloproteinase-5, matrix metalloproteinase-6, matrix metalloproteinase-7, matrix metalloproteinase-8, and matrix metalloproteinase-9, matrix metalloproteinase-10, matrix metalloproteinase-11, matrix metalloproteinase-12, or matrix metalloproteinase-13. Desirable peptides inhibit matrix metalloproteinase-2 or matrix metalloproteinase-9.

At page 6, the amended paragraph beginning on line 29 and continuing on pages 7 and 8:

The invention further provides a method for treating a wound that comprises administering a therapeutically effective amount of a peptide of formula I, II, III or IV :

Xaa₁-Xaa₂-Xaa₃-Xaa₄-Xaa₅-Xaa₆ Xaa₇-Xaa₈-Xaa₉ (I)

Xaa₁₀-Xaa₁₁-Xaa₁₂ Xaa₁₃-Xaa₁₄-Xaa₁₅-Xaa₁₆-Xaa₁₇-Xaa₁₈-Xaa₁₉ (II)

Xaa₁-Xaa₂-Xaa₃-Xaa₄-Xaa₅-Xaa₆-Xaa₇-Xaa₈-Xaa₉-Xaa₁₀-Xaa₁₁-
Xaa₁₂-Xaa₁₃-Xaa₁₄-Xaa₁₅-Xaa₁₆-Xaa₁₇-Xaa₁₈-Xaa₁₉ (III)

Xaa_a-Xaa_b-Xaa_c-Xaa_d-Xaa_e-Xaa_f-Xaa_g-Xaa_h-Xaa_i-Xaa_j-Xaa_k-Xaa_L-Xaa_m-
Xaa₁-Xaa₂-Xaa₃-Xaa₄-Xaa₅-Xaa₆-Xaa₇-Xaa₈-Xaa₉-Xaa₁₀-Xaa₁₁-
Xaa₁₂-Xaa₁₃-Xaa₁₄-Xaa₁₅-Xaa₁₆-Xaa₁₇-Xaa₁₈-Xaa₁₉ (IV)
(SEQ ID NO:20)

wherein:

Xaa₁, Xaa₄, and Xaa₆ are separately each apolar amino acids;

Xaa₂ is a basic amino acid;

Xaa₃ is a cysteine-like amino acid;

Xaa₅ is a polar or aliphatic amino acid;

Xaa₇ is an acidic amino acid,

Xaa₈ is an aliphatic or polar amino acid;

Xaa₉ is an aliphatic, apolar or basic amino acid; and

Xaa₁₀ is a polar, acidic, basic or apolar amino acid;
 Xaa₁₁ is a polar or aromatic amino acid;
 Xaa₁₂ is a polar, basic, aliphatic or apolar amino acid ;
 Xaa₁₃ is an aromatic, aliphatic, polar or acidic amino acid;
 Xaa₁₄ is an aromatic, apolar or polar amino acid;
 Xaa₁₅ is an apolar or acidic amino acid;
 Xaa₁₆ is a basic, a polar or an apolar amino acid;
 Xaa₁₇ is a basic, a polar, an aliphatic, an apolar or an acidic amino acid;
 Xaa₁₈ is an apolar or an aliphatic amino acid;
 Xaa₁₉ is a basic or an aliphatic amino acid;
 Xaa_a is proline;
 Xaa_b is glutamine or glutamic acid;
 Xaa_c is threonine;
 Xaa_d is glycine;
 Xaa_e is aspartic acid or glutamic acid;
 Xaa_f is leucine;
 Xaa_g is aspartic acid;
 Xaa_h is glutamine or serine;
 Xaa_i is asparagine or alanine;
 Xaa_j is threonine;
 Xaa_k is isoleucine or leucine;
 Xaa_l is glutamic acid or lysine;
 Xaa_m is threonine or alanine;
 Xaa_n is methionine;
 Xaa_o is arginine; and
 Xaa_p is lysine or threonine;

wherein the peptide is capable of inhibiting the activity of a matrix metalloproteinase.

At page 9, the amended paragraph 2:

Figure 1 provides a CLUSTAL X (version 1.8) multiple sequence (SEQ ID NOS:2-10) alignment of the cleavage spanning regions of select MMP proenzymes. Figure 1A provides an alignment that highlights conserved residues where an '*' indicates complete identity among the sequences, a ':' indicates 7/9 conserved positions, and a '.' indicates greater than 80% identical positions with mostly conserved substitutions. Figure 1B indicates the positions of heterogeneity in bold.

At page 25, the paragraph beginning on line 9 and continuing on page 26:

In a preferred embodiment (SEQ ID NO:19):

Xaa₁ is proline,
 Xaa₂ is arginine,
 Xaa₃ is cysteine,
 Xaa₄ is glycine,
 Xaa₅ is valine or asparagine,
 Xaa₆ is proline,
 Xaa₇ is aspartic acid,
 Xaa₈ is valine, leucine, or serine,
 Xaa₉ is alanine, glycine or histidine,
 Xaa₁₀ is asparagine, aspartic acid, histidine, arginine, glutamine or glycine,
 Xaa₁₁ is tyrosine or phenylalanine,
 Xaa₁₂ is asparagine, serine, arginine, glutamine, valine or methionine,
 Xaa₁₃ is phenylalanine, valine, leucine, threonine, serine, or glutamic acid,
 Xaa₁₄ is phenylalanine, methionine or threonine,
 Xaa₁₅ is proline or glutamic acid,
 Xaa₁₆ is arginine, asparagine or glycine,
 Xaa₁₇ is lysine, threonine, serine, isoleucine, methionine, glycine, aspartic acid or asparagine,
 Xaa₁₈ is proline or leucine, and
 Xaa₁₉ is lysine, valine or arginine.

At page 27, the paragraph beginning on line 13 and continuing on page 28:

In one embodiment, it is desirable to inhibit MMPs-2 and 9, but to keep the level of MMP-1 relatively unregulated in order to heal chronic wounds. Based on the sequence alignment above one of skill in the art can design a peptide with amino acids that are found in MMP2 and MMP9 proenzyme sequences but not in the MMP1 proenzyme sequence, to produce a peptide that will inhibit MMPs-2 and 9, while leaving MMP-1 uninhibited. Such a peptide is provided by formula IV.

Xaa_a-Xaa_b-Xaa_c-Xaa_d-Xaa_e-Xaa_f-Xaa_g-Xaa_h-Xaa_i-Xaa_j-Xaa_k-Xaa_L-Xaa_m-
 Xaa₁-Xaa₂-Xaa₃-Xaa₄-Xaa₅-Xaa₆-Xaa₇-Xaa₈-Xaa₉-Xaa₁₀-Xaa₁₁-
 Xaa₁₂-Xaa₁₃-Xaa₁₄-Xaa₁₅-Xaa₁₆-Xaa₁₇-Xaa₁₈-Xaa₁₉ (IV)
 (SEQ ID NO:18)

wherein:

Xaa _a is proline;	Xaa ₁ is proline;
Xaa _b is glutamine or glutamic acid;	Xaa ₂ is arginine;
Xaa _c is threonine;	Xaa ₃ is cysteine;

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Xaa_d is glycine;
Xaa_e is aspartic acid or glutamic acid;

Xaa_f is leucine;
Xaa_g is aspartic acid;
Xaa_h is glutamine or serine;

Xaa_i is asparagine or alanine;

Xaa_j is threonine;
Xaa_k is isoleucine or leucine,
preferably isoleucine;
Xaa_l is glutamic acid or lysine,
preferably glutamic acid;
Xaa_m is threonine or alanine;
Xaa_n is methionine;

Xaa_o is arginine;

Xaa_p is lysine or threonine;
Xaa_q is lysine or aspartic acid;
Xaa_r is lysine.

Xaa₄ is glycine;
Xaa₅ is valine or asparagine,
preferably asparagine;
Xaa₆ is proline;
Xaa₇ is aspartic acid;
Xaa₈ is valine or leucine, preferably
leucine;
Xaa₉ is alanine or glycine, preferably
glycine;
Xaa₁₀ is asparagine or arginine;
Xaa₁₁ is tyrosine or phenylalanine,
preferably tyrosine;
Xaa₁₂ is asparagine or glutamine;
Xaa₁₃ is phenylalanine or threonine;
Xaa₁₄ is phenylalanine;
Xaa₁₅ is proline or glutamic acid,
preferably proline;
Xaa₁₆ is arginine or glycine,
preferably arginine;
Xaa₁₈ is proline or leucine,
preferably leucine; and